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Dormancy and after-ripening response of seeds from natural populations and conserved *Physaria* (syn. *Lesquerella*) germplasm and their association with environmental and plant parameters

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ABSTRACT

Seed dormancy studies in *Physaria* are still limited to date. To further understand this trait as well as the after-ripening response in these new crop taxa, we sampled different seed lots of genebank conserved accessions and natural populations of *Physaria fendleri* (syn. *Lesquerella fendleri*) and *Physaria gordonii* (syn. *L. gordonii*) in the U.S. Southwest. We subjected seeds from the natural populations to different after-ripening regimens, storing them over two saturated salt solutions (LiCl and $MgCl_2$) to equilibrate seed moisture levels, at three storage temperatures (5, 25, and 35 °C) for various lengths of time (4, 8, and 12 weeks) and then germinated the seeds at different temperatures (constant 24 °C and alternating 15/25 °C), while seeds from the conserved accessions to 4 and 12 weeks storage at $MgCl_2$ and at an alternating 15/25 °C. Results obtained from the populations indicate significant differences for total germination among storage durations and between germination treatments. In contrast, no significant difference in total germination was found for seeds of the conserved accessions between storage durations, even with gibberellic acid supplementation. We further explored possible associations of the observed germination responses to climatic data and other parameters recorded from the natural populations.

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1. Introduction

Seed after-ripening is necessary to allow seed embryos to overcome dormancy during the development process. The after-ripening process occurs during a period of dry storage of freshly harvested mature seeds, and is essential in releasing dormancy and in promoting germination (Finch-Savage and Leubner-Metzger, 2006; Carrera et al., 2008). Understanding what conditions and factors can influence seed germination behavior during after-ripening, as well as those that can break seed dormancy, has been the focus of numerous studies in new oilseed species (Widrlechner and Kovach, 2000), staple crops (Veasey et al., 2004; Baskin and Baskin, 1998), and weed species (Foley, 2008). Recent studies also have focused on elucidating the genetic regulatory networks controlling this trait, for example the discovery of after-ripening-regulated genes in the model species *Arabidopsis* (Carrera et al., 2008) and transcriptional networks that regulate dormancy (Gao et al., 2012).

Lesquerella (*Physaria fendleri* (A. Gray) O'Kane & Al-Shehbaz) is among the new oilseed crops native to the U.S. and Mexico. It is a predominantly outcrossing species with self-incompatibility and limited interspecific hybridization (Dierig et al., 2001). Lesquerella is being developed for commercial cultivation due to its unusual hydroxy fatty acid (HFA) seed content. *P. fendleri* (also known as *Lesquerella fendleri*) seeds contain lesquerolic HFA, rendering its oil to have similar properties to castor oil. In addition, a number of other valuable uses of lesquerella have been identified including as a potential source of seed meal for livestock feed, and seed coat gum which can serve as a thickening agent for food and industrial products (Dierig et al., 2011; Wu and Hojilla-Evangelista, 2005). Native to the U.S., the species also has documented importance in traditional medicine practices of Native Americans as a cure for various ailments (Elmore, 1944; Swank, 1932).

Because lesquerella is a relatively new cultivated crop, studies on seed dormancy and after-ripening are still limited though there have been experiments on seed dormancy of other related *Physaria* species (Baskin and Baskin, 1990, 2000). Similar to other Brassicaceae such as oilseed rape, the effect of seed storage regimens on lesquerella seed dormancy has not been well investigated (Naeem et al., 2009). The few germination studies in lesquerella include testing the effects of environmental factors such as light and germination temperature (Bass and Clark, 1973; Bass et al., 1966). More recently, we have tested recommended germination protocols prescribed for *P. fendleri* to see if they can be extended

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to other *Physaria* species (Cruz et al., 2012). However, our previous conclusions only apply to seeds from the ex situ genebank that were freshly harvested from regenerated plants. In this study we investigated whether various storage regimens affected the germination of seeds that were freshly collected from natural populations. We likewise explored whether variables from the plants' parental environment correlate with subsequent germination response of lesquerella seeds. The effect of the parental environment on seed germination is a poorly understood area for many plant species, and no previous report was found for this taxa. In other plant species, the effect of the parental environment to germination has been ascribed to changes in seed size and mass that subsequently affect resource allocation (Susko and Cavers, 2008; Luzuriaga et al., 2006; Galloway, 2001), or direct changes in proportion of germinants to avoid competition for limited water (Zeng et al., 2010). Lastly, we compared the germination response of seeds from the wild populations to those that are conserved ex situ and have been in cold storage for more than 5 years. Findings from this study are expected to provide additional information about the seed dormancy and after-ripening behavior of *Physaria* and should have utility in the conduct of germplasm management in the U.S. National Plant Germplasm System (NPGS), as well as for seed handling and propagation of breeding materials during lesquerella cultivar development.

2. Materials and methods

2.1. Natural populations

Plants from *P. fendleri* and *Physaria gordonii* populations were collected in June 2010 from eight localities in New Mexico and Texas where natural populations were found during prior reconnaissance trips in April 2010 (Table 1). Natural plant populations of *P. fendleri* and *P. gordonii* were observed to differ greatly in size and biomass and reproductive development and assigned to different groups. Plants in both groups of the two species attained maturity almost at the same time during seed collecting. This characteristic of *Physaria* populations has been previously reported by Cabin et al. (1997b). We analyzed germination from small plants (<3 g dry weight) and large plants separately. *P. gordonii* is a very close relative of *P. fendleri* and the two were taxonomically separated primarily due to differences in trichome morphology (Rollins and Shaw, 1973).

Various parameters associated with the population sites were collected. The plant density was estimated by examining three gridded areas per site (each grid was 3.72 m^2) and the average number of plants present was determined. The area occupied by the population, as well as the spatial population distribution were recorded (Smith and Smith, 2001). Soil samples were taken from each site and sent to the Soil, Water and Plant Testing Laboratory at Colorado State University, Fort Collins, CO for routine soil testing and sodium adsorption ratio (SAR) analyses. The following parameters from ammonium bicarbonate-diethylene triamine penta acetic acid (AB-DTPA) soil tests were obtained: organic matter (%), pH, electrical conductivity (EC), concentration (ppm) of NO_3-N, P, K, Zn, Fe, Mn, Cu, and SAR. Additional soil data (such as available water supply and water content at various soil depths) were downloaded from the Web Soil Survey of the U.S. National Resource Conservation Service (USDA-NRCS, 2011). Climatic data for each collection site were obtained from the PRISM Climate Group of Oregon State University (http://www.prism.oregonstate.edu), including precipitation, temperature, and dewpoint data for 2009 and 2010. The monthly mean temperature was used to compute seasonal growing degree days (GDD) assuming that all sites shared the growing period of October 2009 to June 2010. The GDD were computed using the below formula with 2.2 °C as base temperature (T_{base}) and 30 °C as the ceiling temperature (Dierig et al., 2012). The maximum temperature (T_{max}) was reset to 30 °C if it was above the ceiling temperature, while the minimum temperature (T_{min}) was reset to the base temperature, if below 2.2 °C. The temperature mean daily range (MDR) for each site was determined by subtracting the 2 year (2009–2010) average low temperature from the average high temperature.

$$\text{Seasonal GDD} = \sum_{June}^{October} \left(\left(\frac{T_{\max} + T_{\min}}{2} \right) - T_{base} \right) \quad \text{(Days in month)}$$

From each population, whole mature plants were harvested and dried to enable the biomass of individual plants to be assessed. The siliques were processed to extract the seeds, and the plant harvest index and 1000-seed weight estimated. The seeds were initially cleaned by hand sieving and by using a seed blower (New Brunswick General Sheet Metal Works, New Brunswick, NJ). The remaining debris was manually removed as well as the green seeds. Green seeds of lesquerella usually fail to germinate although they are already at the mid-maturation stage of seed development (Chen et al., 2009). Seeds from small plants were separately processed and bulked independently of those from large plants.

For the dormancy and after-ripening assay, samples from each population were used in a factorial experiment which included species, plant size, storage temperature (5, 25, and 35 °C), seed moisture content as provided by saturated salt solutions ($MgCl_2$ and LiCl), storage duration (4, 8, and 12 weeks), and germination regimen (constant 24 °C, and alternating 15/25 °C both with light for a 12 h daily period) as factors. Seeds were stored over the salt solutions in sealed 500 ml wide-mouth jars (Nalgene, Rochester, NY) to equilibrate seed moisture. At the different storage temperature, $MgCl_2$ produces relative humidities (RH) between 32.5 and 34.6%, while LiCl between 11.7 and 14.0% (Wexler and Hasegawa, 1954). After each storage duration, the seed moisture contents of the samples were determined. To estimate moisture content, fresh seed weight was compared to weight after drying at 103 °C for 17 h (ISTA, 1999) using the formula: (FW − DW)/FW × 100, where FW is the fresh weight and DW is the dried weight.

Seed samples from the natural population sites were partitioned into three replicates, each replicate having a minimum of 50 seeds, and planted in clear plastic germination plates (11.25 cm × 11.25 cm × 2.5 cm) on blue blotter papers (Anchor Paper Co., St. Paul, MN). The blotters on the germination plates were moistened with distilled water containing 2 ml/L plant preservative mixture (PPM) (PhytoTechnology Labs., Shawnee Mission, KS). PPM does not affect the rate of germination and was used to help prevent or minimize the growth of bacteria and fungus because of the long duration of the germination study (Guri and Patel, 1998). The plates were randomized and placed in two incubators (Percival Scientific Inc., Perry, IA), one set at constant 24 °C, and another set with alternating temperature (15/25 °C) in 12 h cycle. Both incubators had light (2408 lx) for 12 h corresponding to the high temperature cycle of the set at 15/25 °C. The blotter papers were remoistened with distilled water as needed during the duration of the experiment. Seeds with visible protrusion of radicle of about 1 mm from the seed coat (equivalent to the seed diameter) were considered germinated and were removed from the plates each observation period until the last day of the study. The germinations were recorded daily to 45 days after planting (DAP).

2.2. Conserved accessions

Seeds from different seed lots of four conserved accessions previously used by Bass et al. (1966) were obtained from the USDA-ARS National Arid Land Plant Genetic Resources Unit, Parlier, CA. The

Table 1
Locations of collection sites of *Physaria* species and associated population parameters and seasonal growing degree days.

Site	Acc no.	State	County	Long	Lat	Elevation (m)	Population area (m^2)	Plant density (plant/m^2)	GDD[a]
P. fendleri									
1	PARL 859	NM	Socorro	−106.91	34.13	1424	988	1.39	5211
3	PARL 860	NM	Lincoln	−105.97	33.73	1666	1643	3.85	4920
4	PARL 861	NM	Eddy	−104.43	32.91	1041	1466	11.71	5814
12	PARL 869	TX	Culberson	−104.81	30.94	1208	473	2.78	6272
13	PARL 870	TX	Presidio	−104.03	30.31	1444	315	0.95	6078
15	PARL 871	TX	Pecos	−102.91	30.90	929	700	1.21	6569
19	PARL 874	TX	Jeff Davis	−104.17	30.79	1668	773	3.68	5669
P. gordonii									
6	PARL 863	TX	Hudspeth	−105.11	31.74	1141	1597	1.26	6234

[a] Seasonal GDD from monthly mean temperature values (October 2009–June 2010).

different seed lots represented samples from various regeneration periods and have been in cold storage from four to more than 10 years (Table 2). The seeds were prepared and subjected to treatments following the study design used for seeds of natural populations with the same number of replicates and number of seeds per replicate, but limiting the storage periods to 4 and 12 weeks, at $MgCl_2$, and incubation at 15/25 °C only, due to small quantity of available seeds. The seeds were germinated in plates either with or without an aqueous solution of 100 ppm gibberellic acid (GA_3).

2.3. Statistical analysis

The general analyses of variance using arcsine transformed germination data, specific contrasts, and computation of pairwise correlations among soil-related, climate-related and plant and population-related quantitative variables were done using JMP v.7 (SAS Institute, Cary, NC). The time to 50% germination (t_{50}) of viable seeds, and time between 25% and 75% germination (U_{7525}) indicating germination uniformity were computed using the Germinator software's curve fitting module v. 1.02 (Joosen et al., 2010).

3. Results and discussion

3.1. Natural populations

The soils where the populations were collected generally have a sandy clay texture, alkaline nature (pH = 8.1 ± 0.1) with very low organic matter (1.7 ± 0.7%), very high lime estimates (>2%), and an average SAR of 0.66. The populations sampled were located on elevations from 929 to 1668 m, with occupied areas of 315 to 1643 m^2. The plant density in each population was sparse (1–12 plant/m^2) and the plants often exhibited a clumped spatial dispersion pattern (Table 1). The population sites had a computed seasonal GDD ranging from 4920 (site 3 in Lincoln county, NM) to 6569 (site 15 in Pecos county, TX), with an average GDD of 5846. The temperature mean daily range (MDR) varied from 15.39 °C (site 15 in Pecos county, TX) to 19.68 °C (site 6 in Hudspeth county, TX), with an overall average of 17.80 °C. The MDR was determined to have a slight negative association with GDD, which is expected for a continental climate and reflects the huge temperature difference in the areas considered (plot not shown).

We were not able to determine the proportion of small to large plants in each population, only for the plants we collected. The contrasting plant sizes noted in each of these populations may have resulted from differential germination, varying growth rate due to habitat variation or plant vigor, or a result of having a significant portion of the population being kept in soil seed banks. Seed banks of lesquerella have been reported by other studies, are hypothesized to function as an insurance policy to ensure survival during extreme environmental conditions, and there were indications that the seed bank populations could be genetically different from the above ground populations (Evans and Cabin, 1995; Cabin, 1996). It remains to be determined whether the small and large plants we collected are genetically distinct groups. Tissue samples were stored for a future genotyping study.

3.1.1. Comparison among plant sizes, biomass and species and corresponding germination response based on respective groupings

The average whole plant dry weight of large plants from the wild populations was 12.41 g, while for the small plants was 1.54 g (Table 3). These plant dry weights are considerably lower than those of improved *Physaria* varieties, which can have up to 60–80 g dry weight as reported by Dierig et al. (2006). The highest average plant dry weight was recorded on plants from site 6 (31.32 g) followed by site 15 (20.75 g) and site 4 (17.32 g). The site with the lowest average plant dry weight was site 6 (0.48 g) followed by site 3 (0.76 g). Significant differences were found, using *t*-tests comparing large and small plant dry weights, on all sites except sites 15 and 19. The computed harvest index of large plants ranged from 0.05 to 0.14 in plants from sites 3 and 12, respectively. In contrast, the computed harvest index of small plants ranged from 0.01 to 0.14 in plants from sites 4 and 6, respectively. Only from site 4 was a significant difference between harvest index of large and small plants found, suggesting that the plant productivity was affected in this native population by plant sizes. In terms of 1000 seed weight, there was no significant difference between large and small plants. On the average, 1000 seeds from large plants weigh 0.54 g, while those from small plants 0.51 g. The heaviest seeds from large plants were from sites 19 and 12 with 0.65 g and 0.61 g per 1000 seeds, respectively, while for small plants the heaviest were from site 12 with 0.59 g per 1000 seeds.

Table 2
Available passport information of the conserved *Physaria* accessions used by Bass et al. (1966) and specific seed lots used representing different harvest periods.

Acc no.	Species	State	County	Seed lots used[a]	Other ID/info
PI 279650	*P. fendleri*	NM	–	Original, 2002, 2004, 2005	Barclay 998
PI 293016	*P. fendleri*	TX	Hudspeth	Original, 2002, 2003, 2005	19992
PI 299142	*P. gordonii*	AZ	–	2000, 2006, 2008	
PI 302490		AZ	Pima	Original, 2002, 2003, 2006, 2005	Mixture of *P. gordonii* and *P. palmeri*

[a] Seed lots noted as 'original' do not have available information on the actual year of seed increase but was the oldest among the seed lots used.

Table 3
Comparison between dry weight, harvest index and 1000 seed weight of large and small plants in the natural populations.

Site	Plant dry weight (g)		Harvest index		1000 Seed weight (g)	
	Large plants	Small plants	Large plants	Small plants	Large plants	Small plants
P. fendleri						
1	10.12 ± 1.03a	2.85 ± 0.57b	0.10 ± 0.02a	0.12 ± 0.06a	0.53 ± 0.06a	0.41 ± 0.11a
3	13.21 ± 1.32a	0.76 ± 0.12b	0.05 ± 0.01a	0.05 ± 0.01a	0.55 ± 0.01a	0.55 ± 0.04a
4	17.32 ± 3.54a	1.77 ± 0.31b	0.09 ± 0.03a	0.01 ± 0.00b	0.50 ± 0.04a	0.53 ± 0.04a
12	5.40 ± 0.70a	0.90 ± 0.15b	0.14 ± 0.02a	0.11 ± 0.05a	0.61 ± 0.01a	0.59 ± 0.06a
13	6.68 ± 1.00a	2.15 ± 0.52b	0.11 ± 0.01a	0.08 ± 0.01a	0.49 ± 0.05a	0.46 ± 0.04a
15	20.75 ± 7.55a	1.81 ± 0.89a	0.12 ± 0.03a	0.12 ± 0.06a	0.53 ± 0.04a	0.48 ± 0.01a
19	7.49 ± 2.13a	0.79 ± 0.14a	0.05 ± 0.02a	0.11 ± 0.05a	0.65 ± 0.04a	0.51 ± 0.04a
P. gordonii						
6	31.32 ± 6.16a	0.48 ± 0.13b	0.12 ± 0.02a	0.14 ± 0.05a	0.49 ± 0.01a	0.50 ± 0.05a
Mean	12.41 ± 0.97a	1.54 ± 1.18b	0.10 ± 0.01a	0.09 ± 0.01a	0.54 ± 0.02a	0.51 ± 0.02a

Note: Values are mean and standard error. Different letters denote significant differences on germination values from seeds of large and small plants per site using Student's t, $\alpha = 0.05$.

The analysis of variance did not reveal any significant difference between germination parameters (% total germination, t_{50}, and U_{7525}) of seeds from small plants compared to those from large plants (Table 4). Looking at comparisons between plant sizes in each site for germination however, significant differences were found between seeds from the size groups in three of the eight sites (sites 1, 3 and 6). Seeds from large plants in these sites had higher total germination (Table 5). Sites 1 and 3 have lower seasonal GDD values than the other sites and further study of collections from these sites in subsequent seasons is recommended to gather additional data on variability from different years of seed production (Andersson and Milberg, 1998). If this is found to be consistent, the size groups in these populations may exhibit differential fitness due to disparities in total seed germination. In native plants, variation in seed dormancy is ecologically significant and may result in contrasting ecotypes following many generations of selection and altered life history (Casas et al., 2012; Allen and Meyer, 2002; Templeton and Levin, 1979).

Table 4
Analysis of variance summary for germination parameters of freshly-harvested *Physaria* seeds from natural population sites.

Source[a]	DF	Sum of squares	Mean square	*F* ratio	Prob > *F*
Total germination					
S	7	4.913	0.702	17.652	<.0001
P	1	0.009	0.009	0.220	0.639
SL	1	0.008	0.008	0.195	0.659
ST	2	0.346	0.173	4.353	0.0135
GT	1	0.446	0.446	11.210	0.0009
SD	2	5.254	2.627	66.064	<.0001
SL * ST	2	0.665	0.333	8.366	0.0003
SL * GT	1	0.099	0.099	2.491	0.115
SL * SD	2	0.134	0.067	1.679	0.188
ST * GT	2	0.038	0.019	0.477	0.621
ST * GT	4	2.087	0.522	13.121	<.0001
GT * SD	2	0.657	0.329	8.263	0.0003
t_{50}					
S	7	883.539	126.220	18.884	<.0001
P	1	10.113	10.114	1.513	0.219
SL	1	21.527	21.527	3.221	0.074
ST	2	77.406	38.703	5.790	0.0033
GT	1	148.937	148.937	22.283	<.0001
SD	2	175.254	87.627	13.110	<.0001
SL * ST	2	31.090	15.545	2.326	0.099
SL * GT	1	47.431	47.431	7.096	0.0080
SL * SD	2	49.245	24.623	3.684	0.0260
ST * GT	2	70.544	35.272	5.277	0.0055
ST * GT	4	47.567	11.892	1.779	0.132
GT * SD	2	169.664	84.832	12.692	<.0001
U_{7525}					
S	7	857.565	122.509	10.872	<.0001
P	1	12.330	12.330	1.094	0.296
SL	1	24.878	24.878	2.208	0.138
ST	2	98.277	49.139	4.361	0.0134
GT	1	292.782	292.782	25.982	<.0001
SD	2	334.237	167.118	14.830	<.0001
SL * ST	2	25.492	12.746	1.131	0.324
SL * GT	1	14.227	14.227	1.263	0.262
SL * SD	2	44.314	22.157	1.966	0.141
ST * GT	2	138.826	69.413	6.160	0.0023
ST * GT	4	122.040	30.510	2.708	0.0300
GT * SD	2	201.185	100.592	8.927	0.0002

[a] S: site, P: plant size, SL: saturated salt, ST: storage temperature, GT: germination temperature, SD: storage duration.

Table 5
Observed percent germination of seeds collected per site and comparison between seeds from varying plant sizes of *P. fendleri* and *P. gordonii*.

Site	Percent germination[a]		
	Overall mean	Large plants	Small plants
P. fendleri			
1	37.26 ± 2.90	43.35 ± 3.85a	31.17 ± 4.16b
3	48.10 ± 3.69	60.83 ± 2.94a	35.00 ± 6.14b
4	19.37 ± 2.97	16.35 ± 2.49a	22.40 ± 5.40a
12	62.01 ± 2.56	58.10 ± 3.40a	65.92 ± 3.75a
13	49.84 ± 3.45	50.28 ± 4.92a	49.40 ± 4.90a
15	54.84 ± 3.49	54.29 ± 4.15a	55.38 ± 5.68a
19	50.48 ± 3.04	54.14 ± 4.11a	46.52 ± 4.46a
P. gordonii			
6	26.89 ± 2.79	37.31 ± 3.86a	16.46 ± 3.22b
Mean	43.27 ± 1.24	46.13 ± 1.74a	40.39 ± 1.75b

[a] Values are mean and standard error. Different letters denote significant differences on germination values from seeds of large and small plants per site using Student's *t*, $\alpha = 0.05$.

The mean germination observed following harvest, considering all samples from the populations, was 43.27%. The low observed total germination in freshly harvested mature seeds from natural populations indicates the presence of dormancy, reported to be common in seeds of many weedy species (Taylorson and Brown, 1977; Cabin et al., 1997b). Among the seeds from the populations, those from site 12 (Culberson county, TX) were observed to have the highest percent germination with 62.01%, while seeds from site 6 (Hudspeth county, TX) had the lowest with only 26.89% (Table 5). Plants from site 6 are of *P. gordonii* and seeds of this species were observed to have more pronounced dormancy than *P. fendleri*, which is consistent with observations in previous studies (Cruz et al., unpublished data).

3.1.2. Effect of different seed moisture contents and storage temperatures on the germination parameters

The average moisture content of *Physaria* seeds at the start of the experiment was estimated to be at 5.34%. After 4 weeks of storage, this decreased to 4.18% and 5.15% when placed over LiCl and $MgCl_2$, respectively. The average seed moisture continued to decrease when stored over LiCl while remained relatively constant at $MgCl_2$ (Table 6). The final average seed moisture after 12 weeks of storage over LiCl ranged from 3.13 to 4.49%, while 4.75 to 5.78% in $MgCl_2$.

Table 6
Calculated *Physaria* seed moisture content (MC) in the different seed storage parameters.

Storage period (week)	Saturated salt	Storage temperature (°C)	Moisture content (%)
0			5.34
4	$MgCl_2$	5	5.42
		25	5.19
		35	4.85
	LiCl	5	4.79
		25	4.08
		35	3.66
8	$MgCl_2$	5	5.42
		25	4.86
		35	4.81
	LiCl	5	4.74
		25	3.62
		35	3.83
12	$MgCl_2$	5	5.78
		25	5.11
		35	4.75
	LiCl	5	4.49
		25	3.52
		35	3.13

The total germination of seeds stored at LiCl was found to be significantly lower compared to those stored at $MgCl_2$ (39.19% vs. 47.32%), considering all storage temperature regimens (Table 7). In addition, it took significantly longer for seeds stored at LiCl to reach 50% germination (t_{50} = 5.87 vs. 4.66 days in $MgCl_2$), and with less uniform germination as indicated by U_{7525} values (4.18 vs. 3.23 days in $MgCl_2$). Only in samples from sites 1, 6, and 12 were significant differences in the total germination detected, sites 1 and 4 on t_{50}, and sites 4 and 19 on U_{7525}.

Segregating the data based on storage temperature, there were marked differences in total germination among the temperature regimens. Values observed from seeds stored at 5 °C and 35 °C were significantly different from those stored at 25 °C. The average total germination of seeds from these storage temperatures were 45.28% (5 °C), 37.48% (25 °C), and 46.99% (35 °C). The t_{50} values among the three storage temperatures were significantly different: 6.22 days (5 °C), 5.36 days (25 °C), and 4.17 days (35 °C). In contrast, only the U_{7525} values of seeds stored at 35 °C are significantly different from those at 5 and 25 °C, the U_{7525} values being 4.35 (5 °C), 4.07 (25 °C), and 2.68 (35 °C). The higher average germination observed when seeds were stored at 35 °C indicates that warm temperature is very effective in breaking dormancy in *Physaria*. The results parallel those found for leafy spurge, where the most effective after-ripening treatment was at 30 °C storage (Foley, 2008). This likewise mirror the warm conditions in natural habitats of *Physaria* where seeds are dispersed from the plant in late spring and then exposed to summer temperatures before germination in the fall.

Table 7
Average values of total germination, t_{50}, and U_{7525} of lesquerella seeds from natural populations at the different storage and germination regimens.

	Total germination	t_{50}	U_{7525}
Saturated salt			
LiCl	39.19a	5.87a	4.18a
$MgCl_2$	47.32b	4.66b	3.23b
Storage period (week)			
4	39.52a	6.49a	5.58a
8	34.38a	5.58b	3.79b
12	55.32b	3.88c	1.94c
Storage temperature (°C)			
5	45.28a	6.22a	4.35a
25	37.48b	5.36b	4.07a
35	46.99a	4.17c	2.68b
Germination temperature (°C)			
24	35.13a	5.90a	4.40a
15/25	51.41b	4.69b	3.10b

Table 8
Detailed comparison of total seed germination of the collected *Physaria* after seed storage at 4, 8 and 12 week periods.

Site	4 weeks	8 weeks	12 weeks
P. fendleri			
1	47.37 ± 3.16a	20.45 ± 4.00b	43.96 ± 5.77a
3	55.12 ± 5.97a	35.02 ± 6.95b	53.61 ± 5.64a
4	12.82 ± 2.83a	10.58 ± 3.60a	34.72 ± 6.74b
12	57.60 ± 3.40a	52.76 ± 4.52a	75.67 ± 3.97b
13	34.52 ± 5.29a	46.71 ± 5.48a	68.29 ± 5.16b
15	38.08 ± 5.19a	53.76 ± 5.95b	74.17 ± 4.68c
19	43.20 ± 4.05a	45.75 ± 4.44a	61.86 ± 6.24b
P. gordonii			
6	23.90 ± 3.62a	18.71 ± 4.58a	38.05 ± 5.40b
Mean	39.52 ± 2.05a	34.38 ± 2.04a	55.32 ± 2.04b

Note: Values are mean and standard error. Different letters denote significant differences on germination values comparing the various storage durations per site using Student's t, $\alpha = 0.05$.

3.1.3. Comparison of germination parameters between the two germination temperatures

There were significant differences in total germination between germination assays conducted at alternating temperatures (15/25 °C) and constant temperature (24 °C). Individual comparisons in each group indicate that the final germination was significantly higher when lesquerella seeds were germinated at 15/25 °C than at constant 24 °C (51.41% vs. 35.13%) (Table 7). The results support previous findings that an alternating temperature regimen is needed to break seed dormancy in lesquerella (Bass et al., 1966). An alternating germination temperature is usually effective in breaking seed dormancy because it simulates the corresponding diurnal variation in soil temperature (Batlla et al., 2007; Holm et al., 1997; Martinez-Ghersa et al., 1997). In addition to the higher total germination at 15/25 °C, seeds germinate in less time and the germination occurs in a more uniform manner as indicated by significantly lower values of t_{50} (4.69 days vs. 5.9 days in 24 °C) and U_{7525} (3.10 days vs. 4.4 days in 24 °C).

3.1.4. Effect of storage duration on germination

Comparison of the overall germination response after the three storage periods indicated that germinations after 12 week being significantly higher to those stored at 4 and 8 weeks. The average germination after 12 week storage for all sites combined was 55.32% compared to that from shorter storage durations with 39.52% (4 weeks) and 34.38% (8 weeks) (Table 7). This positive response to after-ripening parallels the observations on a relative *Physaria* species, *Physaria ludoviciana*, where increased germination rates were observed after 6 months of after-ripening (Jernegan-Grant et al., 2008). It likewise support the idea that *Physaria* seeds have a non-deep physiological dormancy (Offord and Meagher, 2009; Baskin and Baskin, 2004).

Regarding specific comparisons within sites, improvement in total germination after 12 weeks of storage was observed in seeds from all sites except on seeds from sites 1 and 3 (Table 8). The highest germination after 4 weeks of storage was in seeds from site 12 (57.60%). The poorest performance at 4 weeks was in seeds from site 4, with only 12.82% of the seeds germinating. At 8 weeks of storage, the highest germination was observed in the seeds from site 15 (53.76%), while the lowest was from site 4 (10.58%). After 12 weeks of storage, the highest germination was again observed in seeds from site 12 (75.67%) among those from the other sites. The number of wild populations with seeds exhibiting germinations above 50% totaled two at 4 weeks (sites 3 and 12), two at 8 weeks (sites 12 and 15), and increased significantly to seven after 12 weeks (sites 3, 9, 12, 13, 15, 16, and 19). The results suggested that storing seeds for a period of time can help improve the total germination of freshly harvested materials and agreed with observations on other weed species like *Puccinellia* (Tarasoff et al., 2007) and *Bromus* (Del Monte and Dorado, 2011) as well as other arid zone species (Commander et al., 2009). The increase in total germination was not only observed in seeds stored for longer periods of time in warm temperatures (25 and 35 °C), but also in those at 5 °C (35% after 4 weeks storage compared to 73% after 8 weeks) suggesting that cold dry stratification might have helped release dormancy in lesquerella (see also Table 7 for summary).

Results of analyzing t_{50} values of seeds stored at the different duration correlate with those presented previously using the total germination values. The computed t_{50} of viable seeds among the different storage periods were found to be significantly different in the populations. Smaller t_{50} values, indicating a faster overall germination, were observed after 12 weeks of storage. The t_{50} was 6.49 days at 4 weeks of storage then decreased with longer storage duration; 5.57 days after 8 weeks and 3.88 days after 12 weeks (Table 7). Similarly, a more uniform germination was noted corresponding to a longer storage period as indicated by the decrease in U_{7525} values through time. At 4 weeks storage the U_{7525} was 5.58 days, 3.79 days after 8 weeks of storage, and just 1.93 days after 12 weeks.

3.1.5. Association of germination to environmental factors and population parameters of parental plants

Among the climatic variables, population parameters (such as plant density and population size), and the total germination observed indicate that there are significant correlations in 35 of the variable pairs. Positive correlations were found among total germination, harvest index, seed size, seed weight and soil water content and availability, while negative among germination, population density and population size, plant dry weight and precipitation. None of these correlations however tested significant. In addition, no significant correlation was found between the observed total germination and the other population and environmental parameters enumerated, except to soil EC ($r = -0.80$). Soil EC is strongly associated to soil texture and particle size and is known to be related to other soil properties and crop yield. Sandy soils have lower EC than those with high silt and clay components (Grisso et al., 2009). The negative correlation supports previous reports that lesquerella thrives better in well drained and sandy soils on xeric environments (Rollins and Shaw, 1973).

Among the other parameters analyzed, there were significant correlations between harvest index (HI) of large plants with water availability at 100 cm ($r = 0.72$), GDD ($r = 0.75$), and average maximum temperature ($r = 0.86$). The seed yield and production of dry matter in lesquerella was previously shown to be highly dependent on available water during the flowering stage, as well as the temperature range during the growing season (Dierig et al., 2006; Hunsaker et al., 1998). Among the set of environmental variables there were significant correlations between GDD and elevation ($r = -0.74$), GDD and soil K ($r = 0.80$) as well as between GDD and soil Mg ($r = -0.74$).

Variation in germination in other species has been associated with habitat characteristics (Baskin and Baskin, 1998; Bai et al., 1997) and in some instances may follow a clinal variation (Tieu et al., 2001). This interpopulation variation has been reported in a related species, *P. ludoviciana*, where germination rates were described to be directly affected and vary by the population's location (Beach et al., 2001). In particular, the environmental condition where the mother plant has been exposed to can have a marked effect on the germination behavior of their offsprings, but this has still to be fully understood in many species (Andersson and Milberg, 1998; Evans and Cabin, 1995). Others presuppose that apart from environmental factors influencing dormancy and germination, sib competition maybe more important in determining

Table 9
Effect of gibberellic acid and storage duration on seed germination of conserved *Physaria* accessions stored at $MgCl_2$.

Accession	GA_3 treatment	Storage duration (weeks)	Germination[a]		
			Total (%)	t_{50} (days)	U_{7525} (days)
PI27650	GA_3	4	72.54a	2.56b	1.36b
		12	65.95a	2.76b	1.79ab
	No GA_3	4	63.90a	3.68a	1.59ab
		12	68.85a	4.18a	2.05a
PI293016	GA_3	4	90.98a	3.04b	1.31a
		12	86.55ab	2.93b	1.43a
	No GA_3	4	81.55b	3.93a	1.02a
		12	79.35b	4.02a	1.30a
PI299142	GA_3	4	76.90ab	2.54b	1.09a
		12	85.03a	2.61b	1.42a
	No GA_3	4	65.63bc	4.05a	1.07a
		12	60.02c	4.16a	1.48a
PI302490	GA_3	4	60.53a	4.03a	3.05a
		12	48.83a	3.91a	2.82a
	No GA_3	4	52.89a	4.39a	1.49b
		12	32.65b	4.55a	1.98b

[a] Mean comparison per accession within columns by Student's t, $\alpha = 0.05$.

these two related traits (Kobayashi and Yamamura, 2000). Only in one soil parameter we did find a significant association of *Physaria* total germination and none with other environmental and geographic parameters, as well as population related variables such as plant density in this study. This lack of significant relationship between population parameters and climatic gradients has been reported on other plant species (Clauss and Venable, 2000). There was no significant association between the total germination and seed weight and size in all of our samples. This is in contrast with what was described in other species, where germination fractions highly correlate with seed size – those with bigger seeds have higher germination fractions than populations with smaller seeds (Jurado and Flores, 2005; Bai et al., 1997). A controlled study in the greenhouse or growth chamber may need to be conducted using other accessions with substantial variation in seed size to further verify this in lesquerella.

3.2. Conserved accessions

In the accessions conserved ex situ (Table 2), differences between the total germination across seed batches after storage were observed in PI 302490, PI233016, and PI27650. A lower total germination after 12 week storage period was observed. The t_{50} among seeds lots of each accession vary significantly as well as the U_{7525} values. The lower germination observed in older seed batches is expected and frequently attributed to seed aging in seeds of other plant species (Martinkova et al., 2006; Clark and Moore, 1993; Baskin et al., 1993). Seed aging results to changes in cell membrane properties resulting to poor viability and vigor (Sveinsdóttir et al., 2009; Khan et al., 2004).

3.2.1. Effect of gibberellic acid on Physaria germination

Overall, the application of GA_3 increased the total germination of all seed lots significantly by almost 10% compared to without GA_3 (Table 9). The average total germination of seeds of the accessions with GA_3 is 76.14% compared to 66.23% without. In addition, there is shorter time to reach t_{50} with GA_3 application, compared to without (3.12 vs. 4.13 days). These observations are consistent with results seen in a previous study on other lesquerella species (Cruz et al., 2012; Puppala and Fowler, 2002).

Results from the different seed lots of each accession reflect the aforementioned general observation of positive effects of GA_3, regardless of the age of the lot. Even in the seed lot of PI299142 harvested in 2000, there was an increase of 22% in total germination when GA_3 was used during germination. Among the accessions, the highest average increase in total germination was observed in PI299142, with 18.13% increase across all seed lots, while the least amount of increase was on PI27650 with just 2.87%. In all seed lots, faster germination was noted when GA_3 was applied and this is indicated by lower t_{50} values. The largest decrease in t_{50} was 1.52 days observed in PI299142, which is a *P. gordonii*, when there was GA_3.

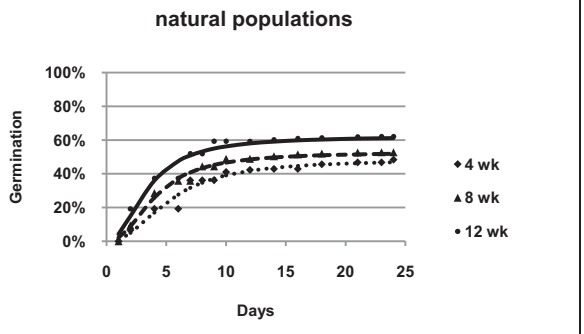
GA_3 has been suggested as a possible seed pretreatment to eliminate the light requirement in *Physaria* seed germination and allow synchronous germination during plant propagation (Puppala and Fowler, 2002). However, GA_3 application may affect the ensuing plant morphology in lesquerella. Plants that developed from seeds germinated with GA_3 may become taller and produce narrower leaves, or have larger plant diameter (Cabin et al., 1997a; Evans and Cabin, 1995). These results however have not been verified yet in the field and could be the subject of a future study. If the effects persist it could be an issue during germplasm characterization and morphological trait evaluation because of altered plant architecture and leaf morphology.

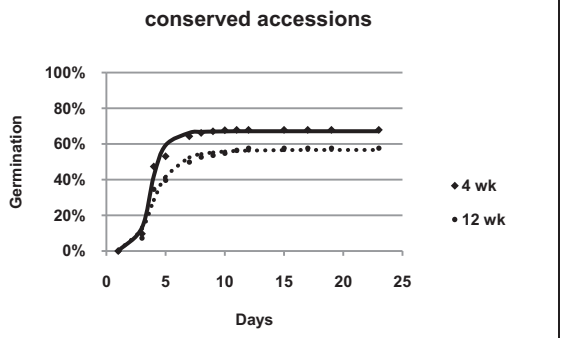
3.2.2. Effect of storage duration

Overall, there was no significant difference observed in the total germination of seeds of the conserved accessions stored at 4 weeks and 12 week periods, regardless of GA_3 application (Table 9). A slight reduction in total germination was observed after taking the seeds from the cold storage and subjecting them to 12 week storage at $MgCl_2$ (Fig. 1). Compared to freshly harvested seeds, the seed lots that have been in storage reached t_{50} at a shorter time and have more uniform germination as indicated by lower U_{7525} values.

There was no consistent trend on the effect of storage duration that can be associated with the different age of the seed lots. However, taking all seed lots of each accession into account, there was an overall reduction in the total germination between the 4 and 12 week storage periods. The highest decrease was in PI302490 (15%) while the smallest in PI27650 (1%).

The above negative effects storage on seeds of the conserved accessions are in contrast to what was determined on the freshly harvested seeds from the natural populations where significant increases in the total germination was observed among the different storage periods. As previously presented, there were slight increases in total germination of fresh seeds in the storage temperatures, including those kept at 5 °C, suggesting that seeds slowly lost dormancy even at cold temperatures. A similar case was reported in





Storage time	germination (%)	t_{50}	U_{7525}	Storage time	germination (%)	t_{50}	U_{7525}
4 wk	50[a]	6.25[a]	5.60[a]	4 wk	67[a]	3.95[a]	1.29[a]
8 wk	45[a]	5.45[ab]	3.65[b]	8 wk	-	-	-
12 wk	61[b]	4.70[b]	2.83[c]	12 wk	58[a]	4.18[a]	1.91[b]

Fig. 1. Comparison of germination curves and germination parameters of seeds from natural populations of *Physaria* and seeds from conserved germplasm at various storage durations germinated without gibberellic acid.

Calendula by Widrlechner (2007) when seeds stored in cold rooms showed consistent increase in germination up to 6.5 years.

4. Conclusions

We have demonstrated that the non-deep physiological dormancy exhibited by seeds from natural populations of lesquerella can be surmounted by storing the seeds in either cold or warm dry conditions. Significant improvements in percent germination of freshly harvested seeds stored for 12 weeks at 32.5–34.6% RH were obtained compared to those kept at shorter durations and lower humidity conditions. This information will be useful in seed production activities in the crop. The higher percent germination obtained by planting the freshly harvested seeds of lesquerella under an alternating temperature (15/25 °C) regimen and by using GA_3 correlates with previous studies in the genus. There was no significant correlation between germination and the climatic, soil, plant and population-related parameters we used in this study, except for soil electrical conductivity. Additional studies will be conducted to further examine the significant differences in germination response between large and small plant groups in three sites to see if these differences are maintained through time.

The after-ripening duration was found directly affecting the time to 50% germination in *Physaria* seeds with less time needed after a longer period of storage, and resulting to more uniform germination. Stored seeds of the cultivated *Physaria* however, did not respond in the same magnitude as freshly harvested seeds suggesting that they could have lost dormancy while in storage for more than 5 years and have started to show effects of aging. The exact range of time when after-ripening will give no additional benefit in increasing the total germination in *Physaria* seed lots remains to be determined.

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